Institut national
de la santé et de la recherche médicale

NeutNet Meeting
Macrophages as targets for neutralization

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de recherches sur le sida

European Union
(NeutNet)

Single cycle of neutralization assay

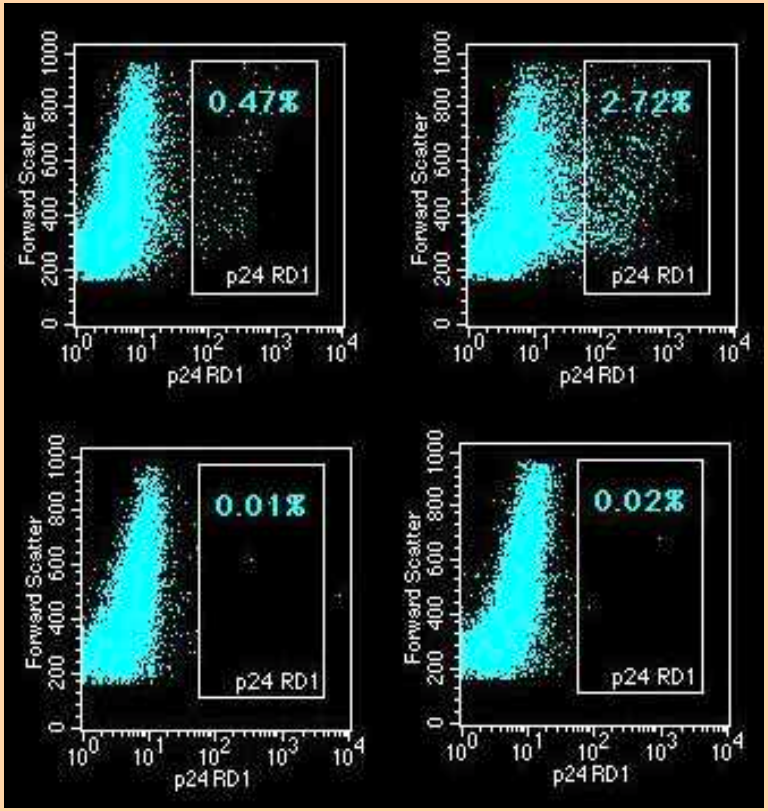
- **Primary cells** : PHA-stimulated PBMCs

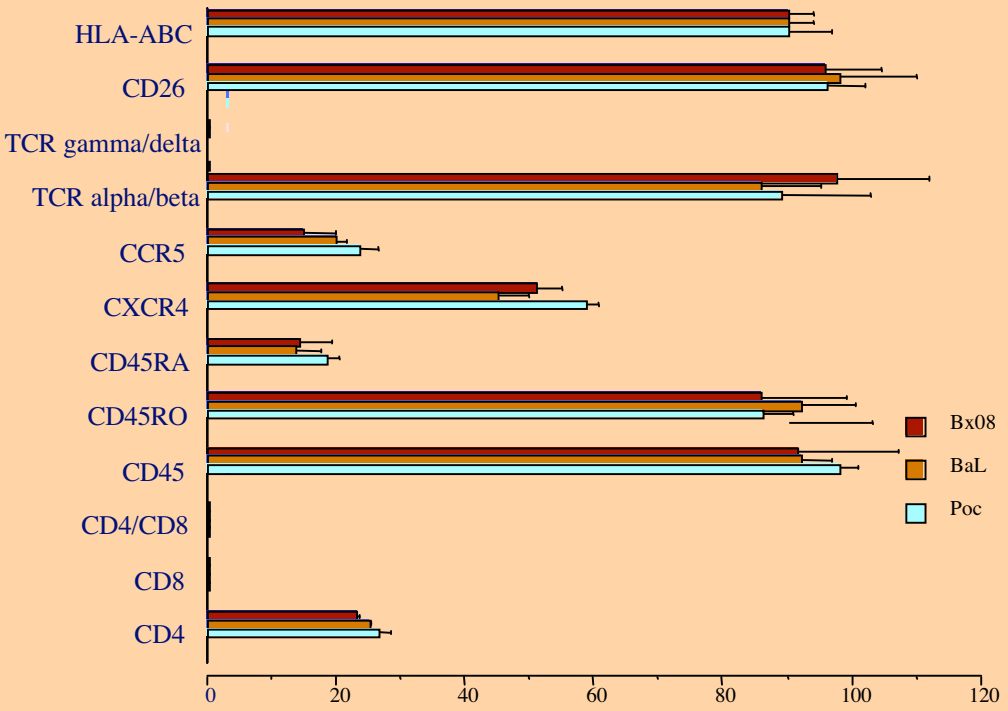
- **Primary isolates** : amplified on PHA-stimulated PBMC and concentrated X80(AMICON 80)

- After 1 day of infection, detection of the **% of infected cells** by dosage of intracellular p24 (flow cytometry)
(J. Mascola, J Virol, 2002)

Advantages: Single cycle of infection
Phenotyping of the infected cells

Flow cytometry analysis of HIV-infected cells after one round of infection using PHA-stimulated PBMCs





HIV-infected lymphocytes subset (% of p24 positive lymphocytes)

The infected cells are CD4+CD45RO+ T lymphocytes

Can neutralizing antibodies block infection of the other HIV target cells ?

⇒ **Main HIV target cells *in vivo* :**

- **CD4+ T lymphocytes**
- **Dendritic cells (DC)**

  Immature DC (iDC) : first targets after mucosal infection

  involved in transport to lymphoid organs, presentation to lymphocytes
- **Macrophages : at the mucosal site, reservoirs**

What is the neutralizing activity of Abs when dendritic cells or macrophages are used as target cells?

Same SOP
Various target cells

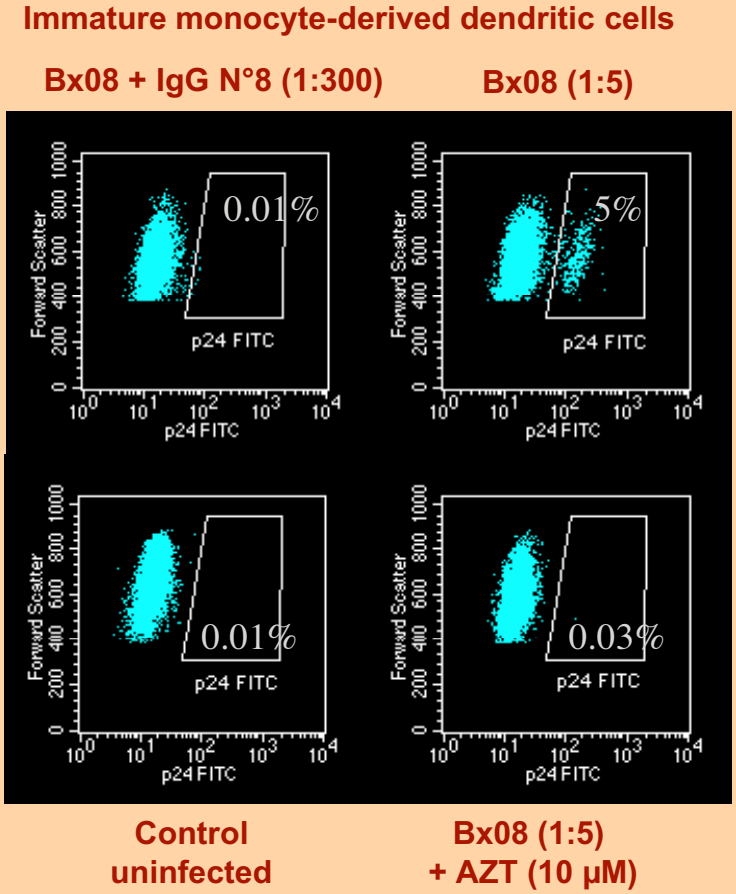
- **Primary cells** :
  - **CD4+ T lymphocytes :** PHA-stimulated PBMCs (Peripheral blood mononuclear cells)
  - **Immature Dendritic Cells** :
    - -monocyte-derived dendritic cells
    - -Langerhans cells differentiated from CD34+ progenitors
  - **Macrophages** : monocyte-derived macrophages

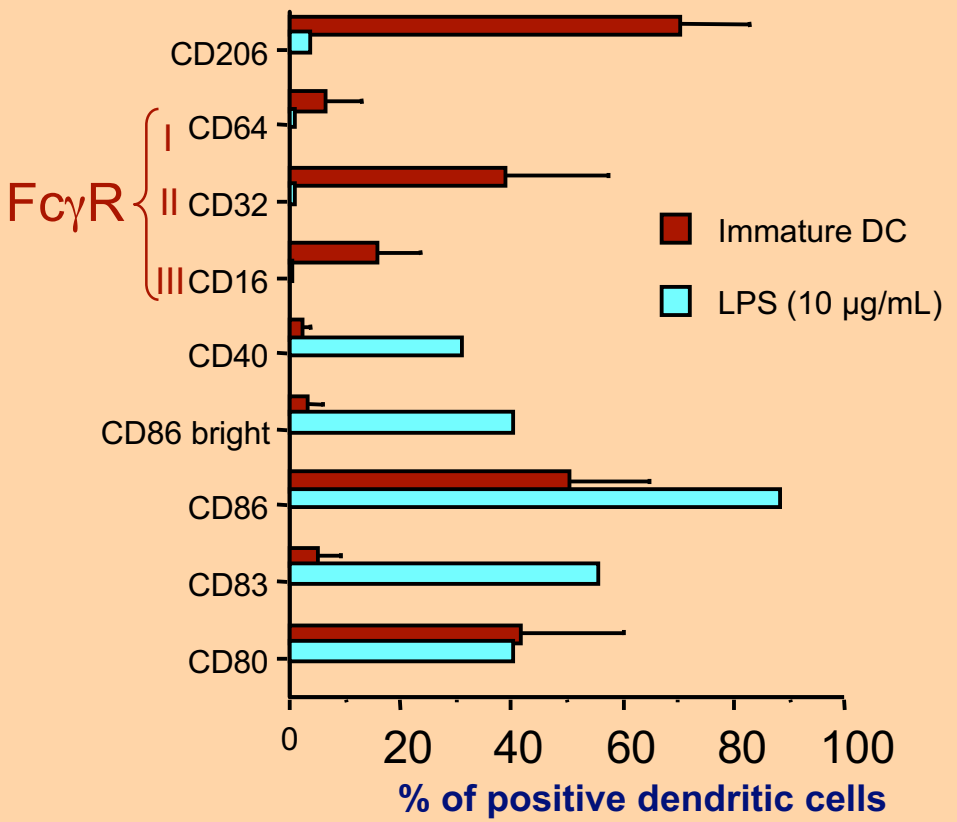
- **Primary isolates** : R5 (BaL, Bx08, TV1) + NeutNet primary isolates

- **% of infected cells** by detection of intracellular p24

Flow cytometry analysis of HIV-infected cells using immature dendritic cells as target cells

Immature monocyte-derived dendritic cells



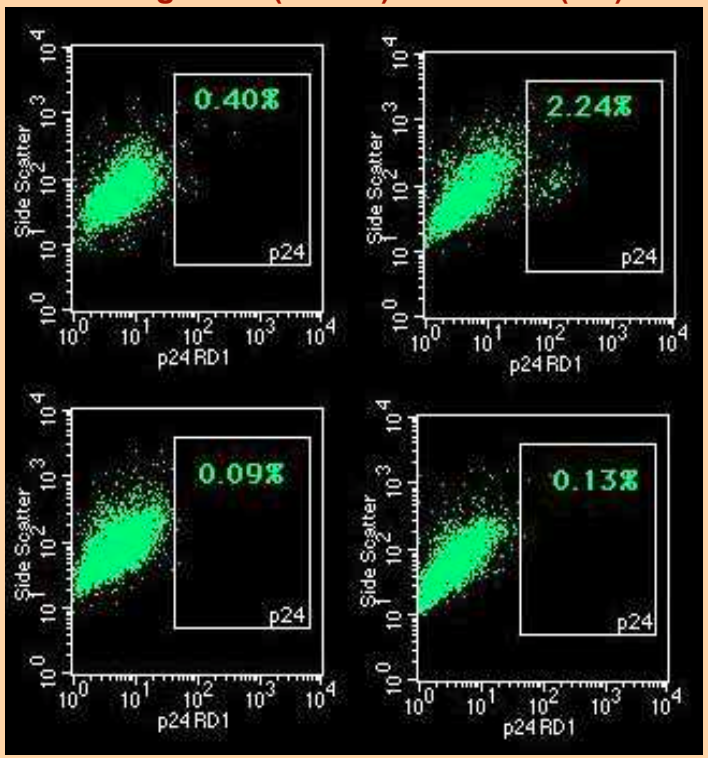


No maturation after infection of immature DC, 40% express CD32

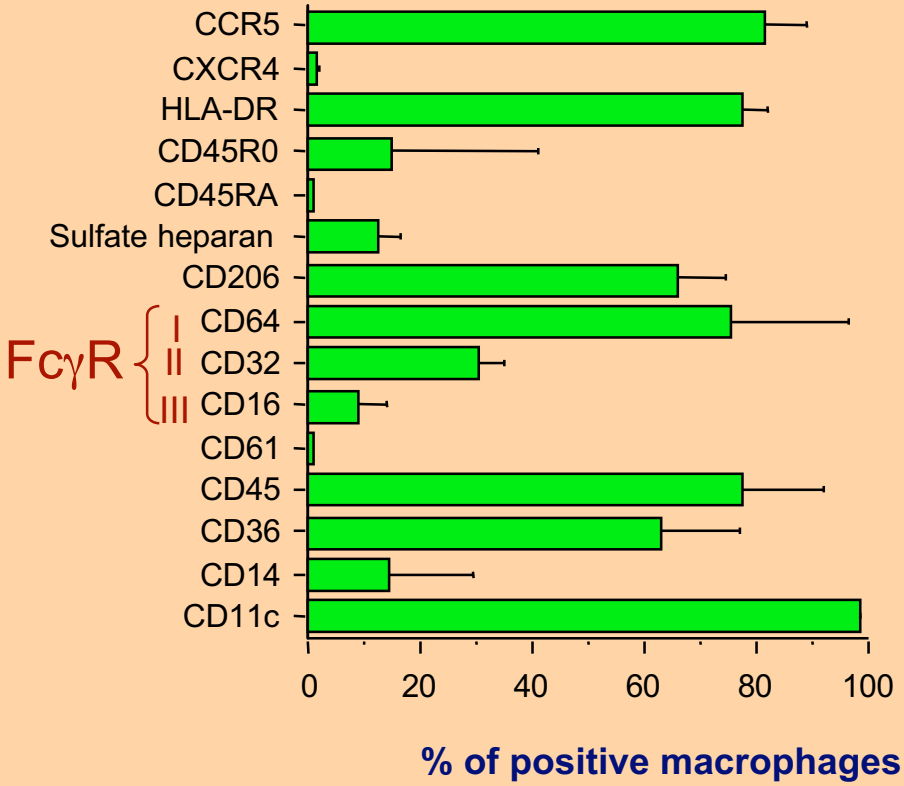
Flow cytometry analysis of HIV-infected cells after one round of infection using macrophages

Monocytes-derived macrophages (MDM)

Bx08 + IgG N°8 (1:5000) **Bx08 (1:5)**



Control uninfected **Bx08 (1:5) + AZT (10 µM)**



The infected cells are macrophages, 80% express CD64

Neutralizing activity of Antibodies on CD4 T Lymphocytes, dendritic cells and macrophages

⇒The 5 well known Neutralizing MAbs: => Increased inhibitory activity

CD4 T lymphocytes < dendritic cells < macrophages

*(J. Immunol. 2004, **173**, 6274-83, Blood 2006, **107,** 4466-4474)*

Inhibitory activity of the NMAbs
(IC90% concentration (µg/ml) that inhibit by 90% the infected cells)

Cells	Primary isolates	NMAbs 2F5	4E10	2G12	IgG1 b12	447-52D
CD4 T cells	BaL	30	50	20	25	50
	Bx08	40	60	100	50	50
	TV-1	50	100	>100	>100	>100
MDDC	BaL	1	5	1	1	2
	Bx08	2	3	2	2	5
	TV-1	6	1.5	5	>50	>50
Langerhans	BaL	5	ND	8	3	5
Macrophages	BaL	0.0025	0.06	0.05	0.5	0.5
	Bx08	0.01	0.2	0.1	5	0.2
	TV-1	0.2	0.05	1	>40	>50

Neutralizing MAbs: => Increased inhibitory activity
CD4 T lymphocytes < dendritic cells < macrophages

11-100 µg/ml
1-10 µg/ml
0.0025-0.5µg/ml

Neutralization on Macrophages
IC90

		IC90			
Cells	Virus	477D	4E10	TriMAb	sCD4
CD4 T Cells	VI191	>25	>25	>25	**1**
Macrophages		**0.1**	**0.08**	**0.04**	**1**
CD4 T Cells	SF162	**6**	>25	**2**	**5**
Macrophages		**0.2**	**0.1**	**0.05**	**5**
CD4 T Cells	Br179.11	>50	>25	>25	>25
Macrophages		>25	**0.5**	>5	**25**

Inhibitory activity of MAbs able to bind to native envelope

Ref. N°	Name			Epitope	*CD4 T cells*	*Macrophages*
ARP 301	221	IgG1	gp 120	gp160/ gp120 (aa 482-495) C term of gp120	>50	20
NIH 857	F105	IgG1k		conformational gp120	>100	>50
NIH 7369	654-30D	IgG1l		tertary gp120	nd	Ni
ARP 3119	CA13	IgG1		cross reactive to env	nd	Ni
ARP 390	ICR39.13	IgG2b		conformational (gp120)	Ni	Ni
ARP 3041	11/68b	IgG1		gp120 (V1, V2+C4)	Ni	Ni
ARP 3036	8/64b	IgM	V3 loop	V3 (aa 300-315)	nd	Ni
ARP 3038	10/540.w	IgG1		V3 (aa 311-321)	Ni	Ni
ARP 3039	8/38	IgG2a		V3 (aa 300-315)	nd	Ni
EVA 331	178.1.1	IgG2ak		V3 (to KSIRI sequense)	Ni	Ni
EVA 3047	IIIB-V3-13	IgG1		V3 (IRIQRGPGRAFTIGC sequence)	>50	3
ARP 3023	257-D IV	IgG1 ?		V3 (KRIHI sequence)	>9.4	0.05
ARP 3024	268-D IV	IgG1 ?		V3 (HIGPGR sequence)	>14	1
EVA 3056	MN215	IgG1		V3 KS/GIHIGPGKAFYTTGEI sequence)	>125	10
NIH 7625	F425B4a1	IgG1 ?		V3	>33	0.4
	391-D	IgG1k		V3	20	1
NIH 7626	F425B4e8	IgG1k		base of V3 loop	25	1
NIH 2534	4G10	IgG1		V3 (RIQRGPGRAFVTGK)	nd	Ni
ARP 324	CRA3	IgG2a	V2	conformational (V2 and C1)	Ni	Ni
ARP 325	CRA4	IgG1		conformational (V2)	Ni	Ni
ARP 3075	62c	IgG1		conformational V2	Ni	Ni
ARP 3218	697D	IgG1 ?		V2 (conformational, region 164-194)	>43	>20
	847-30	IgG1 ?	C2,4,5	C2	nd	>50
ARP 388	ICR38	IgG2b		C4 (aa427-436)	Ni	Ni
	858-D	IgG3 ?		C5 (aa 495-516)	>100	>50
	1331A	IgG3 ?		C5 (aa 495-516)	>100	>50
	450-D	IgG1 ?		C5 (aa 503-509)	>100	>50
	722-D	IgG1k		C5 (aa 503-509)	nd	>50
	670D	IgG1 ?		C5 (aa 503-509)	>100	>50
EVA 3055	GP68	IgG1	CD4 bs	CD4 binding site	Ni	Ni
ARP 3220	654D	IgG1 ?		CD4binding site (discontinious)	>25	>20
	570-D	IgG1 ?		CD4binding site	>100	>50
	654-D	IgG1 ?		CD4binding site	>100	>50
ARP 3078	1.7B	IgG1		CD4 induced	>50	>50
ARP 3079	4.8D	IgG1		CD4 induced	>50	>50
NIH 6882	5F3	IgG1 ?	gp 41	gp41 (526-543)	nd	>100
	181-D	IgG1k		gp141 (I)	>100	>50
	240-D	IgG1k		gp41 (aa 579-604) (I)	>35	1.8
	246-D	IgG1k		gp41 (aa 579-604) (I)	>100	0.8
	50-69	IgG1 ?		gp41 (aa579-613 conformational) (I)	>100	0.4
NIH 7623	F240	IgG1k		gp41 (aa 592-606)	>100	0.5
	98-6D	IgG1k		gp41 (aa 644-663) (II)	>100	>50
	126-6	IgG1k		gp41 (aa 644-663) (II)	>100	>50

11-100 µg/ml

1-10 µg/ml

0.0025-0.5µg/ml

Non-neutralizing antibodies inhibit HIV infection on macrophages or dendritic cells

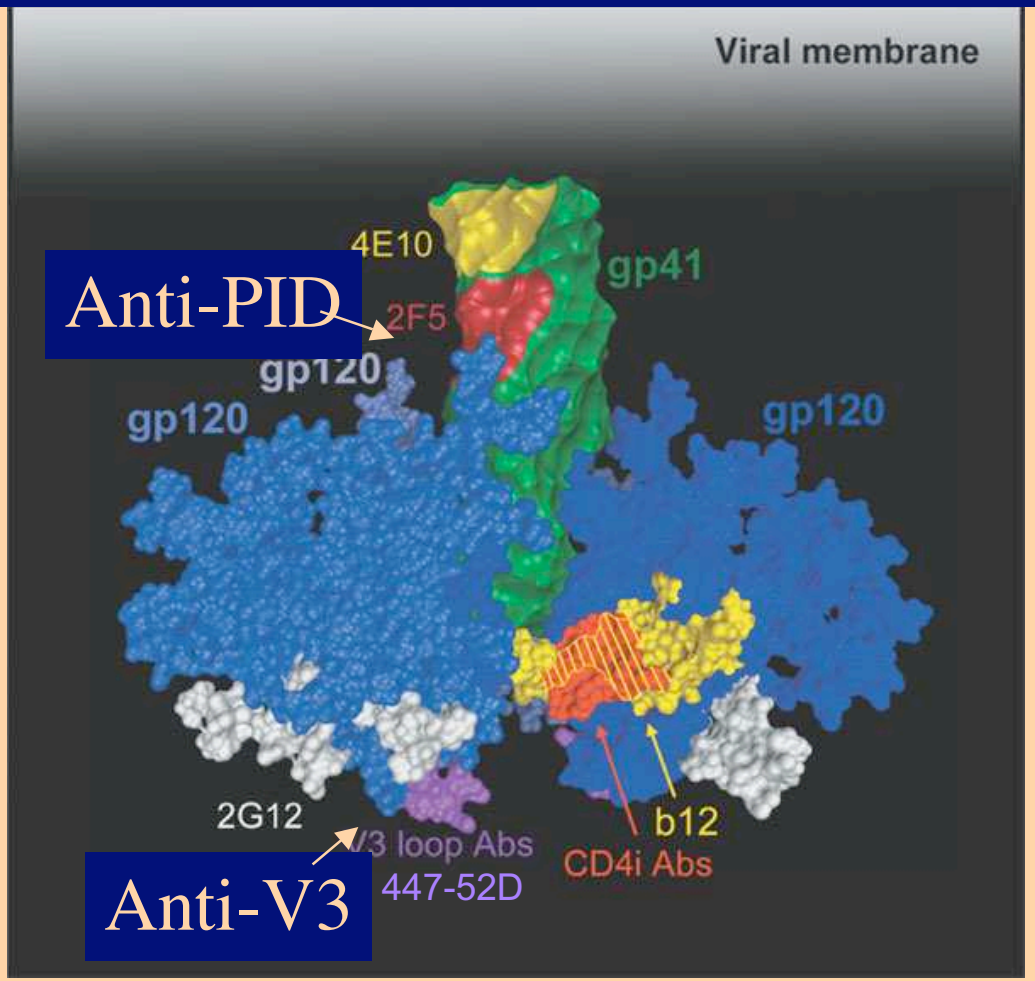
Name	Epitope		IC90 (µg/ml) Macrophages BaL	Bx08	TV1	iDC BaL	CD4 T Cells BaL	Bx08
221	gp160/ gp120 (aa 482-495)	gp120	20	50	>100	nd	>50	nd
IIIB-V3-13	V3 (IRIQRGPGRAFTIGC)	V3 loop	3	nd	nd	nd	>50	nd
257-D IV	V3 (KRIHI sequence)		0.05	0.3	>9	10	>9.4	nd
268-D IV	V3 (HIGPGR sequence)		1	0.5	>15	15	>14	>14
MN215	V3 (KS/GIHIGPGKAFYTTGEI)		10	40	20	>50	>125	>125
391-D	V3		1	0.8	20	nd	20	nd
F425B4a1	V3		0.4	nd	nd	nd	>33	nd
F425B4e8	base of V3 loop		1	5	>30	4	25	nd
240-D	gp41 (aa 579-604) (I)	gp41	1.8	1.9	0.5	20	>35	>35
246-D PID	gp41 (aa 579-604) (I)		0.8	0.5	0.06	45	>100	>100
50-69	gp41 (aa579-613 conformational)		0.4	0.8	0.6	>50	>100	>100
F240	gp41 (aa 592-606)		0.5	0.6	15	50	>100	nd
1577	gp41 (731-752)		25	100	nd	>100	nd	nd

11-100 µg/ml
1-10 µg/ml
0.0025-0.9µg/ml

*(J. Virol., 2006, **80**,6177-6181)*

Epitopes recognized by the 5 Neutralizing Mab

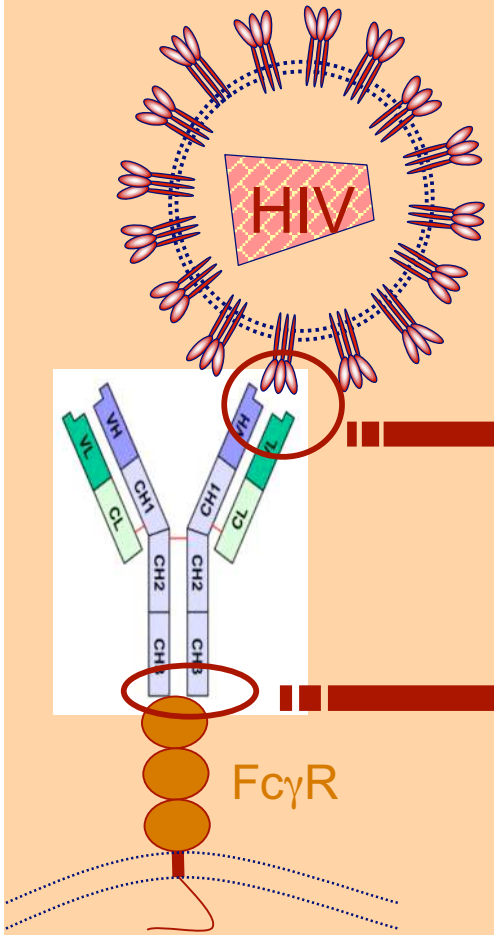
11 «Non-neutralizing» inhibitory MAbs



What is the mechanism of HIV inhibition for these new MAbs ?

From Burton et al.
Nature Immunol, 5, 233, 2004

Mechanism(s) of HIV inhibition on macrophages or dendritic cells



Macrophages and dendritic cells can internalize immune complexes

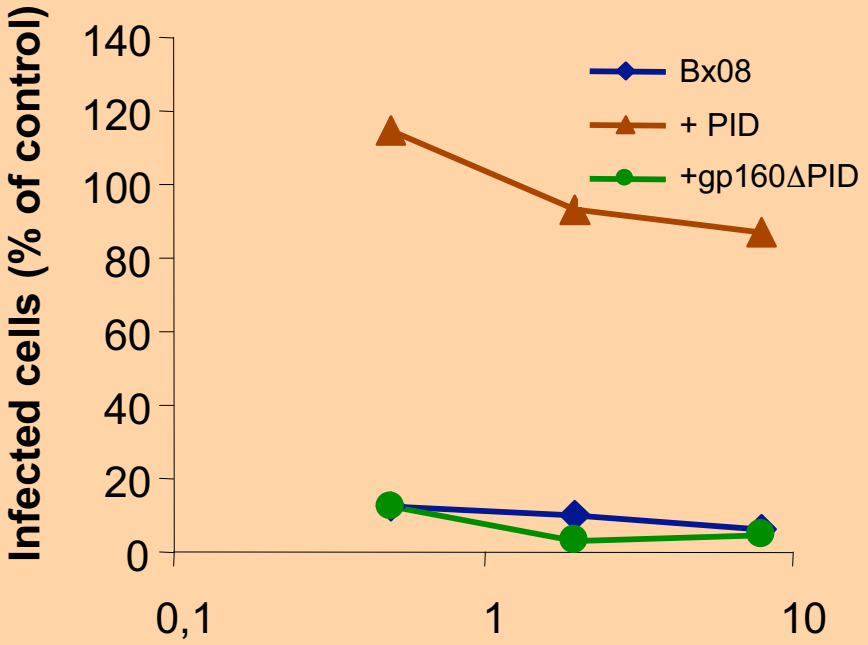
??
Inhibitory activity in presence of gp160 or peptides ?

???
Inhibitory activity in presence of anti-FcγR ?

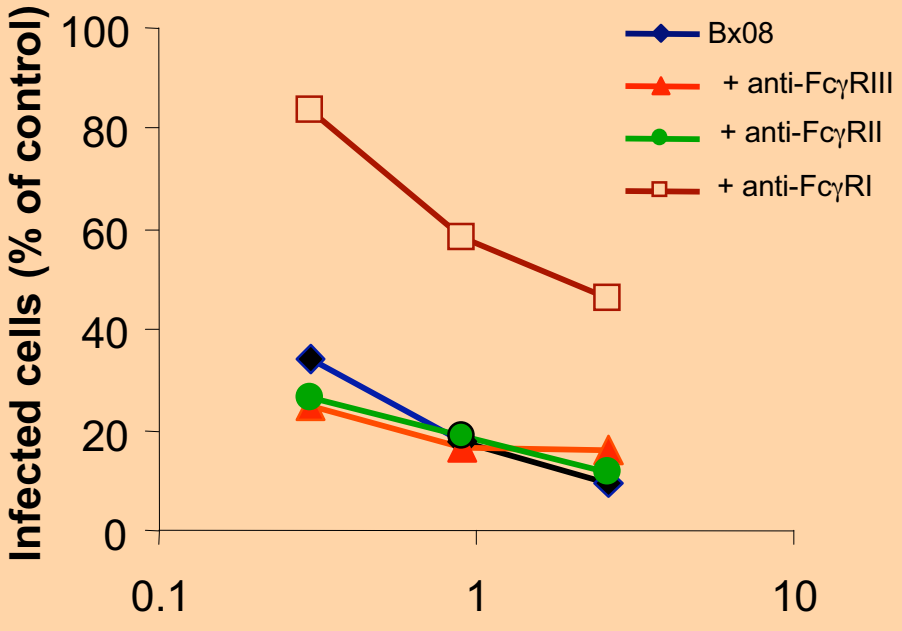
Macrophages : 80 % FcγRI (CD64)
iDC : 45% FcγRII (CD32)

Mechanism of inhibition of «non-neutralizing» Mabs 246-D (anti-PID) on macrophages

Competition with HIV peptides



Competition with anti-FcγR

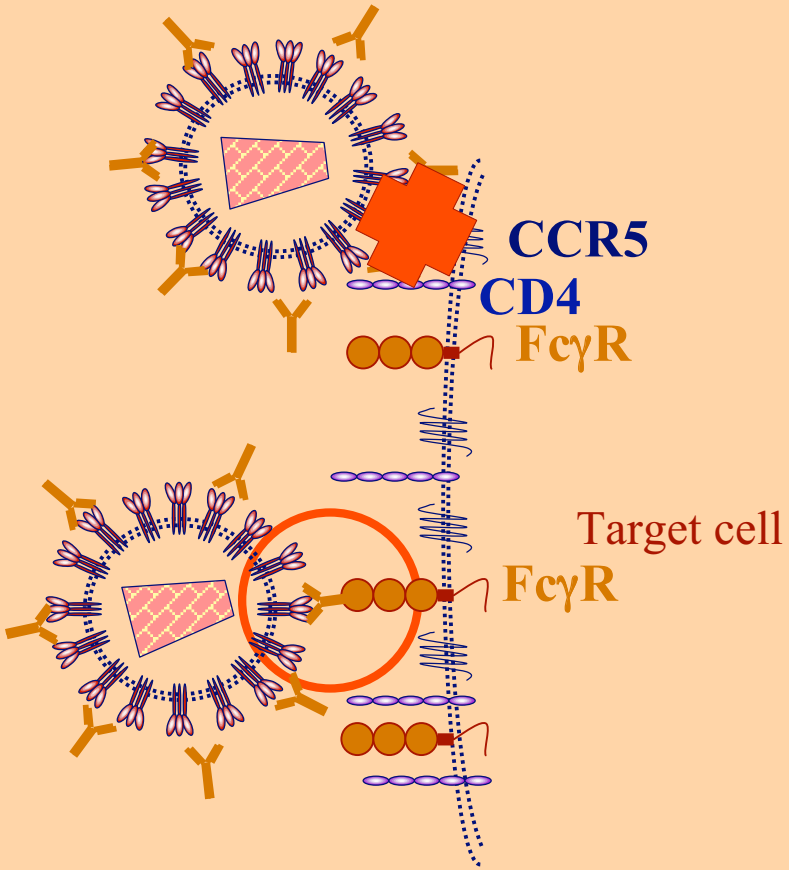


MAb 246-D (µg/ml) anti-PID

Fab part of Ig binds HIV

Fc part of Ig binds FcγRI

Two mechanisms of HIV inhibition by Abs

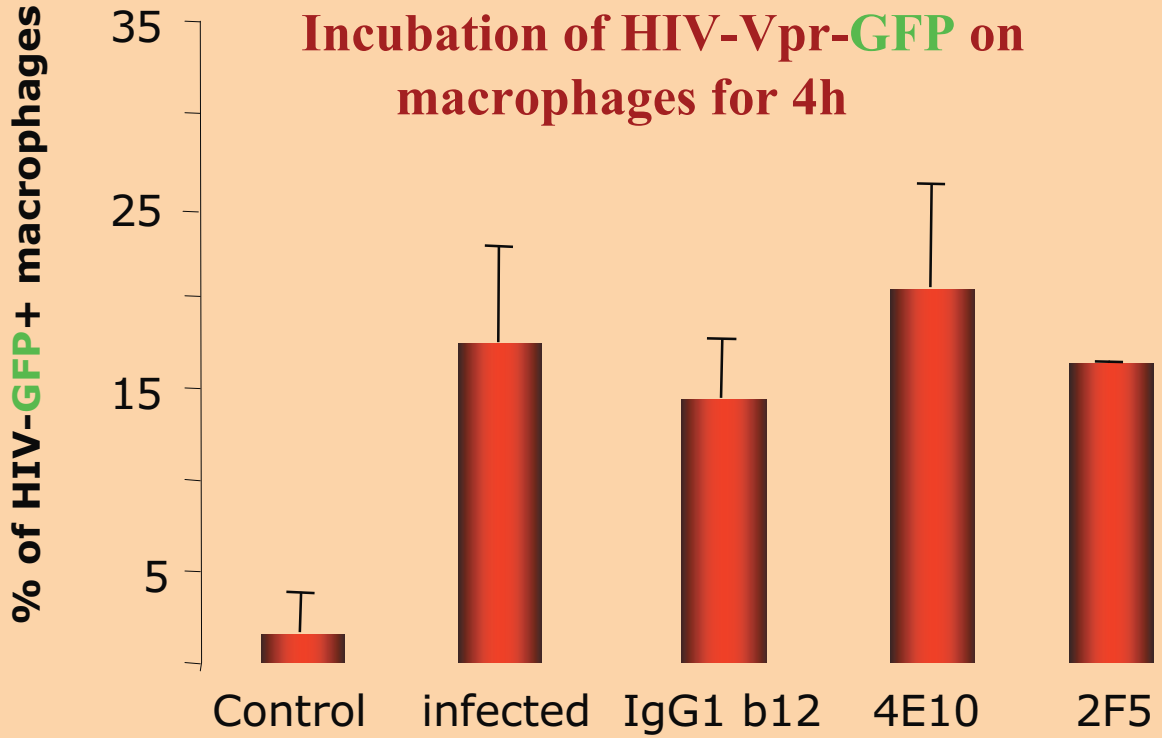


1) - Neutralization of infectivity by Fab or F(ab')$_2$ part of IgG
   - Common for T lymphocytes, DC and macrophages

2) - Inhibition of HIV infection *via* FcγR I (macrophages) or II (DC)
   - Neutralizing and non-neutralizing Abs involved
   => endocytosis and degradation of immune complexes?

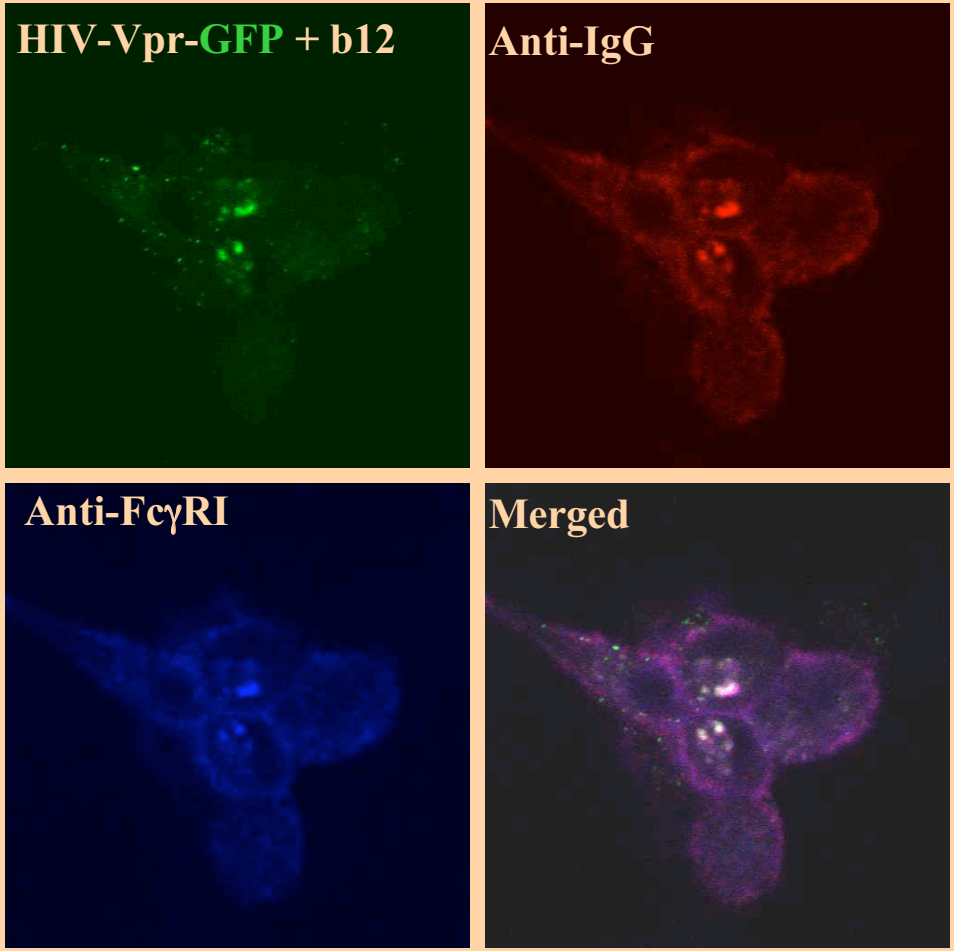
Binding and internalization of HIV in presence of Abs

- **HIV-Vpr-GFP pseudoviruses (env from primary isolates)**
- **Flow cytometry**



Binding and internalization of HIV in presence of Abs

- **HIV-Vpr-GFP pseudoviruses (env from primary isolates)**
- **Confocal microscopy: Infection of macrophages for 4h**



Degradation ?

Conclusion

2 Mechanisms of HIV Inhibition

1) Neutralization of infectivity by Fab or F(ab')$_2$ part of IgG
(Common for CD4T lymphocytes, macrophages and DC)

2) Inhibition *via* FcγR I (macrophages) or FcγR II (DC)
HIV cluster with Ig and FcγR (degradation?)
Involves neutralizing and non-neutralizing inhibitory Abs

- Not detected in "conventional" neutralization assays
- Directed to other epitopes (V3, PID)
- More easily detected in sera from HIV infected individuals,
- Higher inhibitory activity *in vitro*.

=> More easily induced by immunogens?

=> Should be induced by vaccination